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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/500,936	03/25/2005	Ayrookaran J. Poulose	GC717-2-US	1489

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EXAMINER
MOORE, WILLIAM W

ART UNIT	PAPER NUMBER
1656	

MAIL DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/500,936	POULOSE, AYROOKARAN J.	
	Examiner	Art Unit	
	WILLIAM W. MOORE	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 August 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 15-18* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1 and 15-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 18 August 2009 has been entered, amending claim 1 and canceling claims 2 and 3. The amendment of claim 1 clarifies the description of the intended subject matter and overcomes both the objection of record of claim 1 and the rejection of record of claims herein under the second paragraph of 35 U.S.C. § 112, which objection and rejection are WITHDRAWN. Claims 1 and 15-18 remain in the application.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 USC § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1 and 15-18 are rejected under 35 USC § 103(a) as being unpatentable over Estell et al., **US 7,332,320**, and Roggen et al., **US 2005/0181446**, both of record, in view of Bryan et al., **US 4,980,288**, made of record herewith and Hastrup et al., **US 5,741,694**, made of record with Applicant's IDS filed 22 August 2007.

This new grounds of rejection is necessitated by Applicant's amendment of claim 1 that adds the limitation "wherein the variant has improved stability as compared to [the] wild-type GG36". Applicant's arguments at pages 3 and 4 of the Response filed 18 August 2009 have been fully considered but are moot in view of the new grounds of rejection combining the teachings of Bryan et al. and Hastrup et al. with those of Estell et al. and Roggen et al., of record. Applicant's arguments are nonetheless addressed to the extent that they concern the issue of thermostability, particularly the suggestion that "nothing in Estell et al. [ ] would lead one of skill in the art to produce the presently claimed variants that have protease activity in detergents and are thermostable." Yet Estell et al. teach that "[i]n addition to the mutations specifically described herein, the present invention finds use **in combination with mutations known in the art** to effect **altered thermal stability**, altered substrate specificity, modified activity (e.g., modified affinity and/or avidity), modified function, increased specific activity, **and/or altered pH (e.g., alkaline) stability of proteins**" and further teach that "[a]fter the

variants are produced, they can be screened for the desired property (e.g., altered or low or reduced immunogenic response, **increased thermal or alkaline stability**, etc.)", and "[w]hile the instant invention is useful to reduce the immunogenic response produced by a protein, the mutations specified herein find use **in combination with mutations known in the art to result** [in] **altered thermal stability** and/or altered substrate specificity, modified activity, improved specific activity **or altered alkaline stability** as compared to the precursor", where the further mutations produce "one or more substitutions **selected from the group consisting of positions corresponding to . . . 218 . . .** of *B. amyloliquefaciens* subtilisin, as set forth in SEQ ID NO:2" – which is identical to the amino acid sequence of SEQ ID NO:3 herein – and that "[s]uch mutations find use in **. . . modulating the overall stability and/or proteolytic activity of the enzyme**" (emphases supplied). See col. 15, lines 30-36, col. 26, lines 3-15 and 40-50, and the paragraph spanning cols. 26 and 27. Thus one of ordinary skill in the art at the time the invention was made would have recognized that Estell et al. contemplate making allergenicity-altering mutations as well as ancillary, stability modulating, substitutions, such as N218S, in subtilisins generally. Estell et al. then define thermal stability at col. 39, lines 50-59, as "at least about a 5% or greater increase or decrease (in most embodiments, it is preferably an increase) in the half-life of the catalytic activity of a mutant [subtilisin] when exposed to a relatively high temperature and neutral pH as compared to the precursor [subtilisin]", and they specifically teach that introducing the N128S mutation in a subtilisin amino acid sequence will produce a variant with the advantageous property of improved stability and compatible with lower allergenicity in their Example 6 at cols. 51 and 52 where N218S is among a select set of eight "Lower Allergenicity Stabilizing Mutations". Estell et al. also teach how to determine the extent of thermal stability that is conferred by a mutation in a subtilisin amino acid sequence in their Examples 9 and 10, using conditions similar, save the buffer employed and a continuous incubation only at a single temperature, to those described at page 34, lines 2-8, of Applicant's specification.

Structural requirements of claim 1 as amended 18 August 2009 remain unchanged. The specification discloses that the GG36 subtilisin amino acid sequence is the amino acid sequence set forth in SEQ ID NO:6, and this sequence is modified by either a V26T or a V26S substitution, a position identically numbered in both the amino acid sequences of SEQ IDs NOs:3 and 6, and claim indicates that either of the substitutions for valine at position 26 is joined to an N218S amino acid substitution which will occur at position 212 in the amino acid sequence of SEQ ID NO:6 that is identifiable by correspondence to position 218 of SEQ ID NO:3. While Estell et al. teach all of the substitutions recited in claim 1, and teach that the N218S substitution is among a preferred set of stabilizing substitutions that may be combined with, and will be

compatible with, any other of their allergenicity modifying substitution, such as the V26T and V26S substitutions, they do not specifically disclose the preparation of a variant wherein the amino acid sequence of SEQ ID NO:6 herein comprises either of the substitution pairs V26T and N218S or V26S and N218S that are within the scope of claim 1. Thus Roggen et al. are again cited for teaching the preparation of variants of the wild-type "savinase" subtilisin having the amino acid sequence of their SEQ ID NO:24, a sequence that is entirely identical to the amino acid sequence of SEQ ID NO:6 herein, wherein a serine or threonine is substituted for the valine at the subtilisin BPN'-correspondent 26 to provide a variant subtilisin having modified immunogenicity. See, e.g., the "savinase" epitope regions "sav4.0", "sav14.0", "sav.16.0", "sav17.0", and "sav18.2" in Table 2 at pages 54-55, as well as claims 76 and 80-85, particularly claim 80.

Neither Estell et al. nor Roggen et al. teach the extent of thermostability that the N218S substitution confers on a subtilisin, and neither indicates the extent to which the N218S substitution was well-known in the art at the time the invention was made to confer thermostability in subtilisins that have protease activity under detergent wash conditions. Thus Bryan et al. **'288** and Hastrup et al. **'694** are now cited to show that the N218S substitution had long been well-known in the art at the time the invention was made to enhance the thermostability of diverse subtilisins with protease activity in detergent wash conditions by comparison with the thermostability of precursor subtilisins that lack this substitution, as evidenced by the prolonged half life of the catalytic activity of N218S-comprising variant subtilisins after incubations at temperatures that exceed the temperature indicated in Table 4 of Applicant's specification. While Bryan et al. **'288** were not the first to teach incorporating the N218S substitution in a subtilisin – the history of this substitution is discussed in the Conclusion below – they were the first to measure the degree of thermostability this substitution provides in a subtilisin having protease activity under detergent wash conditions. In particular, Bryan et al. **'288** teach that this substitution is advantageously made in subtilisins generally at col. 6, lines 9-23, and that it "enhances the thermal stability of subtilisin" at col. 8, lines 10-20. Bryan et al. **'288** also teach that their singly-substituted GX7150 subtilisin variant – which is the amino acid sequence of SEQ ID NO:3 herein modified only by the N218S substitution – has "almost four times [the proteolytically active half-life] of the wild type" after incubation at 65°C when measured over a period of time exceeding 13 hours and further teach that this "single amino acid change . . . dramatically increases the kinetic thermal stability of subtilisin." See col. 12, lines 36-68, col. 13, lines 38-56, and Figure 4. Bryan et al. **'288** additionally teach that the N218S substitution provides thermally stabilizing advantages in a variety of environments, wherein the catalytic activity of the variant is prolonged relative to the catalytic activity of the wild

type subtilisin in detergents and in actual wash conditions comprising "15 minutes at 75 RPM agitation at 55°C". See col. 13, line 69, through col. 15, line 19, particularly the results of Table I at col. 15, and Figures 5-7. Bryan et al. moreover teach that the N218S substitution is the primary contributor to the thermostability of several multiply substituted subtilisin variants. See col. 15, line 49, through col. 16, line 19, including the results of Table II, and Figure 8.

Because Bryan et al. do not specifically extend their teaching to the introduction of the N218S substitution in the subtilisin having the amino acid sequence of SEQ ID NO:6 herein – termed a GG36 subtilisin in Applicant's specification but also known in the art as subtilisin 309 and marketed under the trade name Savinase™, the teaching of Hastrup et al. **'694** of introducing the N218S substitution in the amino acid sequence of subtilisin 309 is now cited. Numbering the position as 218 by correspondence with the amino acid sequence of subtilisin BPN', which is the amino acid sequence of SEQ ID NO:3 herein, Hastrup et al. **'694** designate their singly-substituted variant as "f) Asn-218 Ser" in the list of mutations at col. 21, and teach that their N218S variant has significantly increased washing ability by comparison with the wild type subtilisin 309 in actual wash conditions comprising the detergent composition and the assay conditions taught at col. 15, line 64, through col. 16, line 44, for a duration of 10 minutes at 100 rpm, providing the results in Table VI of Example 6.2.6 at col. 25, lines 15-40. Hastrup et al. **'694** further teach that their subtilisin 309 variant having only the N218S substitution exhibits significantly enhanced thermostability with the same detergent composition and same duration of wash at 100 rpm when "tested against the wild type enzyme" at 40°C and a two-fold enhanced thermostability by comparison with the wild type enzyme at 60°C in Table VII of Example 6.2.7 at col. 25, lines 41-63.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to prepare a subtilisin variant of claim 1 herein having "protease activity under detergent wash conditions" as well as having "improved stability as compared to [the] wild-type GG36" by selecting the "savinase" amino acid sequence of SEQ ID NO:24 of Roggen et al. identical to the GG36 subtilisin amino acid sequence of SEQ ID NO:6 herein to prepare a variant subtilisin exhibiting an altered immunogenicity by introducing either of the V26S or V26T substitutions that are taught by both Estell et al. and Roggen et al. and to further introduce the N212S substitution, i.e., at the position corresponding to position 218 of SEQ ID NO:3 herein, in order to provide a dually-substituted variant according to claim 1 herein having both protease activity under wash conditions and improved thermostability by comparison with the wild-type subtilisin 309/GG36. This is because (1) Estell et al. identify a region present in subtilisins generally that comprises a valine at the subtilisin BPN'-correspondent position 26 and contributes to the

generation of a T-cell response in animals and teach that replacing the valine in this region with serine or threonine will alter the immunogenicity of the variant by comparison with that of the native subtilisin, (2) Roggen et al. agree with Estell et al. in particularly identifying the valine at the position 26 in SEQ ID NO:6 herein as contributing to an epitope and teaching, as in their claim 80, that it may be advantageously replaced with a serine or threonine, and (3) Estell et al. further teach that the N218S substitution, already well-known in the art, is among a preferred set of stabilizing substitutions advantageously combined with an amino acid substitution that alters the immunogenicity of a variant subtilisin relative to the precursor, and (3) the artisan would readily recognize that the asparagine at position 212 in the amino acid sequence of SEQ ID NO:6 corresponds to the asparagine at position 218 in the amino acid sequence of subtilisin BPN' according to Estell et al., and (4) that the artisan would be well aware that Bryan et al. had already replaced the asparagine with a serine in subtilisin BPN', and Hastrup et al. had already replaced the asparagine with a serine in subtilisin 309/GG36 subtilisin, to provide enhanced thermostability in both subtilisins. It would further have been obvious to one of ordinary skill in the art at the time the invention was made to prepare the DNA molecule, expression vector, host cell, and cleaning composition of claims 15-18 herein because Estell et al. teach (1) that it is advantageous to make such variant subtilisins by preparing DNA molecules encoding such variants, expression vectors comprising such variant-encoding DNA molecules, and host cells comprising such expression vectors in order to practice a method of making such variants utilizing such host cells and (2) that subtilisin variants having altered immunogenicity are advantageously incorporated in cleaning compositions. Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success in practicing the claimed invention.

This application currently names joint inventors. In considering patentability of the claims under 35 USC § 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 USC § 103(c) and potential 35 USC §§ 102(e), (f) or (g) prior art under 35 USC § 103(a).

Conclusion

The prior art made of record herewith, Zukowski et al., **US 4,914,031**, Bryan et al. **US 4,990,452**, Bryan et al., **US 5,116,741**, van Eekelen et al., **US 5,336,611**, Zukowski et al., **US 5,397,705**, and Stabinsky et al., **US 5,399,283**, and not relied upon is considered pertinent to

applicant's disclosure. Stabinsky et al. **'283** have the earliest US priority date, January 1986, for an N218S substitution made in the subtilisin BPN' amino acid sequence, while Zukowski et al. **'031** have an October 1988 US priority date for their disclosure of an N218S substitution in the subtilisin encoded by the *Bacillus subtilis aprA* gene. Like Bryan et al. **'288**, discussed above, Bryan et al. **'452**, Bryan et al., **'741**, van Eekelen et al., **'611**, and Zukowski et al., **'705**, teach advantageous combinations of the N218S substitution with other amino acid substitutions in various subtilisins.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 571.272.0933 and whose FAX number is 571.273.0933. The examiner can normally be reached Monday through Friday between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's Supervisory Primary Examiner, Andrew Wang, can be reached at 571.272.0811. The official FAX number for all communications for the organization where this application or proceeding is assigned is 571.273.8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571.272.1600.

/William W. Moore/
Examiner, Art Unit 1656

/ANAND U DESAI/
Primary Examiner, Art Unit 1656
September 8, 2009